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BRAF-targeted therapy and immune responses to melanoma

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Abbreviations: CCL2, C-C chemokine ligand 2; CCR2, C-C chemokine receptor type 2

Type I BRAF inhibitors and immunotherapy constitute two new exciting approaches for the treatment of advanced malignant melanoma. We have recently elucidated a role for host C-C chemokine receptor Type 2 (CCR2) in the antineoplastic effects of Type I BRAF inhibitors in mice, supporting the therapeutic potential of combining BRAF inhibitors with immunotherapy.

Half of all human melanomas harbor activating mutations in the serine-threonine protein kinase BRAF, most commonly at position V600 (BRAF^{V600}). BRAF inhibitors kill melanoma cells harboring BRAF mutations by interrupting oncogenic BRAF^{V600} signaling through the mitogen-activated protein kinase pathway, which generally supports cell survival and proliferation. The BRAF inhibitors vemurafenib and dabrafenib induce tumor regression in a high proportion of patients bearing BRAF^{V600} mutant metastatic melanoma, and vemurafenib improves overall survival as compared with standard of care chemotherapy.¹ The emergence of drug resistance upon BRAF inhibition was predicted even before oncologists observed disappointing relapses. This has been a common issue with previous targeted agents against chronic myelogenous leukemia (with imatinib, used as an inhibitor of BCR-ABL), gastrointestinal stromal tumors (with imatinib, used to inhibit mutant KIT), non-small-cell lung cancer (with gefitinib, used to inhibit mutant EGFR) and breast

cancer (with trastuzumab or lapatinib, used to inhibit amplified ERBB2/HER2). Multiple mechanisms of resistance to BRAF inhibitors have been discovered, including *NRAS* mutations, *BRAF* amplification, the emergence of BRAF splice variants and downstream alterations in MEK. These have directed the next steps in melanoma research, including the development of approaches to concurrently inhibit BRAF and MEK.²

The contribution of the host to the antineoplastic effects of BRAF inhibitors was poorly understood since—until recently—no murine model of transplantable, syngeneic BRAF^{V600E}-driven melanoma was available. Some patients treated with BRAF inhibitors exhibit increased intratumoral CD8⁺ T cells soon after therapy. This and other data reviewed in ref. 3 suggested that BRAF inhibitors could engage the host immune response to mediate tumor regression. We have now took advantage of two relatively resistant syngeneic variants of BRAF^{V600E}-driven mouse melanoma xenografts and a transgenic mouse model of

melanoma to illustrate the ability of a Type I BRAF inhibitor, PLX4720, to reduce the local production of C-C chemokine ligand 2 (CCL2).⁴ With these models, we demonstrated a key role for host C-C chemokine receptor type 2 (CCR2, the main CCL2 receptor), but not for host CCL2, in the antitumor activity of PLX4720 (Fig. 1). Notably, our melanoma models did not express CCR2, yet clearly some heterogeneity exists with respect to CCL2 production and response to BRAF inhibition across a spectrum of human melanomas (unpublished data). Evidently, multiple host mechanisms might be at play, depending (at least in part) upon the genetic diversity of the tumor. In this light, our data were complementary to recent findings demonstrating the role of oncogenic BRAF^{V600E} in stromal immunosuppression upon the induction of interleukin (IL)-1 secretion by melanoma cells.⁵

A robust increase in the ratio between tumor-infiltrating CD8⁺ T cells and FOXP3⁺ regulatory T cells (Tregs) as well as CD8⁺ T-cell functions were partially

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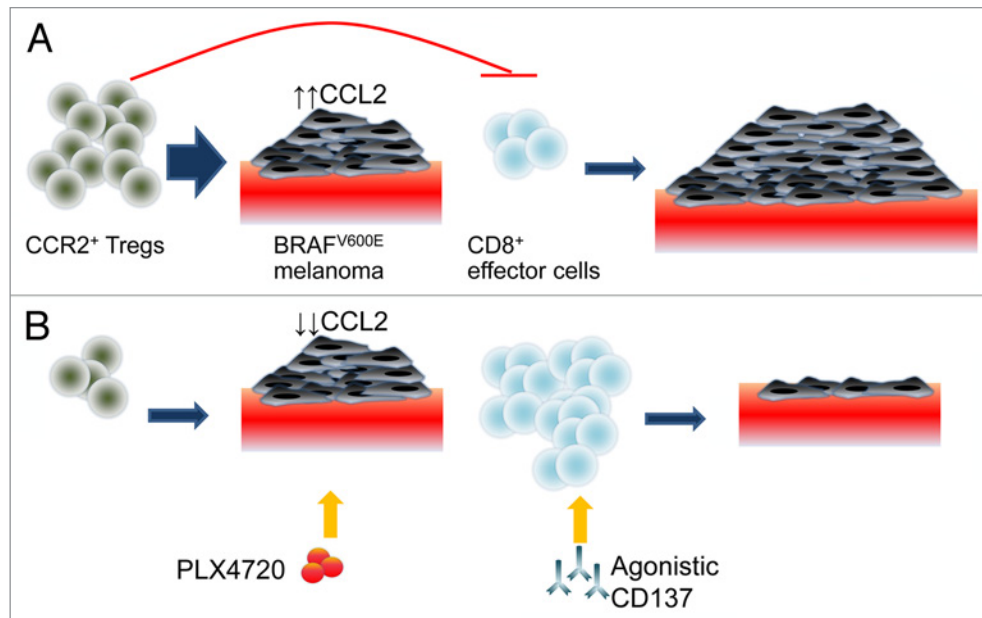


Figure 1. BRAF inhibitors and agonistic CD137-targeting monoclonal antibodies suppress BRAF^{V600E}-expressing melanoma. **(A)** C-C chemokine ligand 2 (CCL2) produced by BRAF^{V600E}-expressing melanoma promotes the accumulation of C-C chemokine receptor type 2 (CCR2)⁺ regulatory T cells (Tregs), limiting the expansion of antitumor CD8⁺ T cells. **(B)** BRAF inhibitors decreased the amount of CCL2 produced by BRAF^{V600E}-expressing melanomas, in turn reducing the local abundance of CCR2⁺ Tregs and increasing the recruitment and/or expansion of antitumor CD8⁺ T cells. Such an expansion of antitumor CD8⁺ T cells can be further enhanced by the administration of agonistic anti-CD137 monoclonal antibodies.

required for the therapeutic activity of PLX4720 (Fig. 1).⁴ A high CD8⁺/FOXP3⁺ T-cell ratio is widely recognized as an indicator of an effective cell-mediated immune response. Although we showed that CCR2 was expressed predominantly on a proportion of tumor-infiltrating CD11b⁺ cells and CD4⁺ Tregs, and that only intratumoral Tregs decreased upon the administration of PLX4720, identifying the exact nature of the host CCR2⁺ cell compartment that underpins the therapeutic efficacy of PLX4720 requires a complex genetic approach involving the specific deletion of *CCR2* in CD11b⁺ myeloid cells or FOXP3⁺ T cells.

These findings, conceptual advances and emerging experiences,^{6–8} and the fact that BRAF inhibitors meet most of the criteria of immunomodulatory agents, warrants the evaluation of combining immunotherapy with BRAF inhibitors, like vemurafenib or dabrafenib, in preclinical mouse models and clinical trials. Therefore, we have progressed to show that the combination of PLX4720 with agonistic anti-CD137 or anti-CCL2 antibodies exerted significant antitumor activity against transplanted and de novo melanomas, in a dose- and schedule-dependent fashion.⁴

To our surprise, no obvious combinatorial activity was noted when BRAF inhibitor was combined with antibodies targeting the cytotoxic T-lymphocyte antigen 4 (CTLA4), programmed death 1 (PD1) or T-cell immunoglobulin mucin 3 (TIM3). Whether these checkpoint inhibitors require different therapeutic schedules or are efficient in other models of melanoma remains to be elucidated. The schedules and doses of PLX4720 were important for the efficacy of the combinatorial regimen involving anti-CD137 antibodies, with PLX4720 to be given preferably first or concurrently. Our data with *Braf*^{CA}*Tyr-creER*^{T2}*Pten*^{fl/fl} mice are the first to demonstrate a single agent activity for an immunotherapeutic approach in this model of de novo melanomagenesis.⁴ Still, the mechanisms underlying the improved therapeutic effects of PLX4720 combined with PLX4720 may be different in *BRAF*^{V600} mutant tumors that exhibit a high sensitivity to BRAF inhibitors.⁹ Given the low frequency of lymphocytes that infiltrate the melanomas of *Braf*^{CA}*Tyr-creER*^{T2}*Pten*^{fl/fl} mice, this transgenic model may be not especially suitable to mimic patients that naturally mount antitumor immune responses.

Based on natural immune responses to melanomas and on the ability of PLX4720 to reduce CCL2 expression by melanoma cells, there is no strong theoretical argument to disregard agents that promote intratumoral CD8⁺ T-cell function. Certainly, anti-CTLA4 (ipilimumab) and anti-PD1/PD-L1 antibodies are making a significant impact in the treatment of malignant melanoma, and these agents are nowadays being evaluated in clinical trials in combination with BRAF inhibitors. The resistance to BRAF inhibitors often leads to increased PD-L1 expression by melanoma cells, providing a strong rationale for combinatorial regimens including anti-PD-L1 antibodies.¹⁰ Adoptive T-cell transfer (ACT) should also be considered in this setting,^{11,12} as BRAF inhibitors have been shown to limit vascular endothelial growth factor (VEGF) production by cancer cells. Unfortunately, the use of ACT is now rather restricted because of the special expertise needed for this type of therapeutic approach. BRAF inhibitors efficiently combine with immunotherapies that mediate antitumor effects via CD8⁺ T cells, and current data support the clinical testing of combinatorial regimens including BRAF-targeted agents and immunotherapy in

advanced melanoma patients. These combinations might be considered as a first line therapy for patients affected by early stage BRAF-mutant melanoma, to potentially achieve a higher proportion of long-term tumor regressions.²

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Disclosure of Potential Conflicts of Interest

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